- INTRODUCTION

DETERMINATION OF PD2 VALUE ON FROG RECTUS ABDOMINIS MUSCLE.

The PD_2 value determination on frog rectus abdominis muscle, measures the potency of drug leading to 50% agonistic effect. The experiment involves isolating the rectus abdominis muscle of frog, maintaining it in organ bath with physiological salt solution and increasing concentrations of a drug. The drug's effect on muscle contraction is recorded, and PD_2 value is determined from the drug response curve (DRC), providing insight into drug's efficacy and potency.
The study of DRC indicates:

1) To record the dose response curve for acetylcholine on isolated frog rectus abdominis muscle
2) To plot the dose versus response and log-dose versus response curves for acetylcholine
3) To understand the concept of threshold concentration, ceiling concentration and half maximal effective concentration (EC50)
4) To determine the PD2 value for acetylcholine on frog rectus abdominis muscle

- EQUIPMENT REQUIRED

Animal :-	Frog
Drug:-	Acetylcholine stock solution (1mg/ml)
Instrument:-	Student Organ Bath, kymograph.
Physiological salt solution:-	Frog Ringer's

- PRINCIPLE

The PD_2 value measures the potency of a drug, representing the negative logarithm of the molar concentration required to produce 50% of the maximum response (EC_{50}). To determine the PD_2 value, the frog rectus abdominis muscle is isolated and mounted in a suitable physiological salt solution. Increasing concentrations of the drug are applied to the tissue, and the contractile responses are recorded. A dose-response curve is constructed by plotting the drug concentration (on a logarithmic scale) against the percentage of the maximum response. From the curve, the EC_{50} is identified as the drug concentration at which 50% of the maximum response is observed. The PD_2 value is calculated as:

$$PD_2 = -\log 10[EC_{50}]$$

This value provides an indication of the drug's affinity and potency on the frog rectus abdominis muscle. The experiment requires careful maintenance of physiological conditions and precise measurements to ensure accurate results.

PROCEDURE:

1) To determine PD_2 value, the frog is sacrificed as per CPCSEA guidelines, its rectus abdominis muscle is isolated.

2) Two rectus muscles are separated from midline and one rectus muscle is mounted in organ bath. One end is connected to isotonic frontal writing lever and other is connected with aeration tube.
3) Tissue is allowed to stabilize for 30minutes and at interval of 10minutes physiological salt solution is changed with fresh ones.
4) Once the tissue is stabilized, the acetylcholine is introduced in increasing pattern to achieve contractile responses. A five minutes time cycle is followed for each response as follows :

00sec : Start the drum and record a base line for 30sec
30sec : Add the drug to the bath organ and take the response to drug for 90 sec.
120sec : Stop the drum and wash
210sec : Give second wash
0sec/300sec : Start the drum and repeat as above.

5. Measure the height of contractions of acetylcholine and tabulated the readings.
6. Determine the dose of acetylcholine which produces 50% response and convert it into molar concentration.
7. Calculate PD_2 value for acetylcholine, $\mathbf{PD_2 = - \log [EC_{50}]}$

CALCULATION & INTERPRETATION:

1. Concentration of Acetylcholine (Ach) (µg/mL): Constant at 100 µg/mL for each entry.
2. Amount Added in Organ Bath: Given in mL
3. Amount Added in Organ Bath (in µg): This will be calculated using the formula

Amount in µg = Conc. Of Ach X Amount Added in mL

4. Concentration of Acetylcholine in µg/mL (in Organ Bath contains 20mL Solution):

Calculated as $\frac{\text{Amount Added (µg)}}{\text{20mL}}$

5. Response (in mm): Newly provided values.
6. % Response

Calculated as : Response / Maximum Response X 100, using the maximum response from the provided.

CONCLUSION

The determination of the PD_2 value on the frog rectus abdominis muscle is a reliable method to evaluate the potency of a drug. By constructing a dose-response curve and calculating the EC_{50}, the PD_2 value provides a quantitative measure of the drug concentration required to achieve 50% of the maximum response. This parameter is essential for assessing drug efficacy and comparing pharmacological agents.
The experiment emphasizes the importance of maintaining physiological conditions, precise measurements, and accurate data analysis to ensure reproducibility and reliability. Overall,

Sr. No.	Conc. Of Ach (µg/ml)	Amount added in organ bath		Conc of Ach in µg/mL (organ bath contains 20 mL sol.	Response (in mm)	%Response	Conc of Ach (µmol/L)	Conc of Ach (µmol/mL)	Log conc
		In mL	In µg						
1.	100	0.1	10	0.5	20	21.98	2.752	0.002752	-2.560
2.	100	0.1	10	0.5	20	21.98	2.752	0.002752	-2.260
3.	100	0.2	20	1	45	49.45	5.505	0.005505	-2.259
4.	100	0.4	40	2	76	83.52	11.010	0.0011010	-1.958
5.	100	0.8	80	4	90	98.90	22.019	0.022019	-1.657
6.	100	1.6	160	8	91	100.00	44.038	0.044038	-1.356

this method serves as a foundational approach in pharmacological studies to understand drug-tissue interactions.

- IDEAL OBSERVATION

RESULT:

- The PD_2 value for acetylcholine on the frog rectus abdominis muscle was determined successfully.
- As acetylcholine concentration increases, muscle contraction intensifies until it reaches a plateau, indicating receptor saturation.
- The results of this procedure depend on several critical factors, including the preparation of the muscle, the stability of the physiological solution, and the accurate measurement of drug concentrations.
- Variations in experimental conditions, such as temperature, pH, and muscle integrity, can significantly affect the outcomes.

DISCUSSION

The determination of the PD_2 value on the frog rectus abdominis muscle involves understanding the drug's potency by quantifying its ability to produce a physiological response. The frog rectus abdominis is commonly used due to its sensitivity to pharmacological agents and the ease with which its responses can be measured in vitro.